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Extraction and Evaluation of the Antimicrobial Activity of Polyphenols from Banana Peels Employing Different Extraction Techniques

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Abstract: Polyphenols are natural antioxidants and play a vital role in inhibiting oxidative stress induced by the body's free radicals. Banana peels are a significant agro-industrial waste. This waste could be utilized to extract polyphenols to process various functional foods and nutraceuticals. An investigation was executed to extract polyphenols from banana peel using the sonication and maceration techniques. Three different polar solvents, methanol, ethanol and acetone, were used at four different concentrations: 25%, 50%, 75% and 100%. Yield (%), Total Polyphenolic Content (TPC), Total Flavonoid Content (TFC) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging assays were performed. The results from the current study articulate that extraction by sonication yields a higher quantity of polyphenols than the maceration technique. The study also concludes that ethanol leads to better extraction than other solvents used in this study.

Keywords: antimicrobial activity; polyphenol; agro-food waste; banana peel; antioxidants; maceration; sonication; ultrasound-assisted extraction; total phenolics; total flavonoids; DPPH; green extraction

1. Introduction

Fruits and vegetables are an essential part of the human diet and are rich in phytochemicals that perform various positive functions in the human body [1–5]. Investigators have originated the banana species *Musa sapientum*, which belongs to the family Musaceae and possesses predominant antiulcer [6], antidiabetic [7], anti-inflammatory [8] and antioxidant properties [9].

The contents of phenolic components are available in the second-highest value in banana peel compared with other peels of fruit such as pineapple, avocado, passion fruit, papaya, melon and watermelon [10]. Over the world, consumption of bananas is high [11]. Different parts of the banana fruit are well recognized in Ayurveda and traditional folklore medicine as dealing with numerous ailments such as diabetes [12,13].

The peel of a banana comprises several antioxidant composites, such as dopamine [14] and gallic catechin [15]. This study proposes that extracts obtained from the peel of numer-

ous banana varieties can be utilized to fight free-mediated radical ailments [16]. The weight of a whole banana fruit includes about 35% peel [17].

Researchers predominately focus on diabetes, which also includes determining active and fresh antidiabetic mediators to separate the biologically active constituents from the sources of herbal medications that are predicted to have the properties of antidiabetics defined in the earliest discoveries [18]. The peel of a banana has a high micronutrient concentration compared to the pulp of the fruit [19]. It receives excessive attention because its antioxidant and nutritional properties, particularly components such as catechin, ascorbate, dopamine and galocatechin [20].

To extract polyphenols from a sample, an ultrasonic bath with pre-set time and temperature mixed the sample with organic solvents such as ethanol, methanol, acetone, etc. It was necessary to create cavitation bubbles near the sample to create sound waves that eventually caused cell walls to rupture, which led to the removal of phenolic compounds from the sample and their subsequent extraction into the solvent medium [2,4,9,21].

It is widespread in laboratories to use ultrasound-assisted extraction due to its efficiency and simplicity [22,23].

The current study was designed to assess the effect of maceration and sonication on the extraction of bioactive compounds from banana peel powder. It also determined the influence of different solvents (ethanol, methanol, acetone) at different concentrations (25, 50, 75, 100%) on extraction parameters.

2. Materials and Methods

Fully mature and ripened Banana fruits were purchased from the local market in Sargodha. The solvents and reagents used in the study were of analytical grade from Merck, Germany, supplied by Merck's Islamabad distributor. Washing fruits was done to remove any dust, dirt, or residues of pesticides, weedicides or fungicides, etc. The peels were separated from the pulp for both fruits and cut into small pieces and dried with a 'Hot Air Oven' at 50 °C for 48 h. With the assistance of 'The Cyclotec Sample Mill,' the dried peels, with moisture content less than 10%, were ground into a fine powder. The peel powder was then put in polyethylene zip bags and it was made sure that the bags were airtight; the bags were kept at refrigeration temperature (4–6 °C).

Proximate Analysis

By following the standard methods of AOAC International, moisture (%), ash (%), crude protein (%), crude fat (%), crude fiber (%) and NFE (%) were determined for banana peel [24].

2.1. Conventional Extraction (Maceration)

The maceration technique extracted polyphenols from banana peel powder following Elfalleh et al. (2012), with few amendments [25]. Preliminary studies were conducted to determine the optimum solvent concentration (25%, 50%, 75% and 100%) and sample to solvent ratio (1:20, 1:15 and 1:10). After preliminary studies, three different solvents were used: acetone, methanol and ethanol at two different selected concentrations: 50% and 75%. The sample to solvent ratio was set to 1:15, and the temperature for the maceration technique was set to 40° C. Three grams of both peels were placed in 250 mL conical flasks separately in triplicate, and 45 mL of solvent was introduced at 50% and 75% concentrations. A 'Shaking Water Bath' (Tecator 1024, FOSS Analytical AB, Pal Anders vag, Hoganas, Sweden) was used in this procedure. The conical flasks were placed in the shaking water bath for about 20 h. After 20 h, all the samples were filtered through Whatman filter paper 41, and centrifugation was performed for about 10 min at 5000 rpm (Beckman J2–21, Brea, CA, USA). Then the solvent was evaporated with a 'Rotary Evaporator' (Buchi Labortechnik, Rotavapor R-300, Meierseggstrasse, Flawil, Switzerland). Evaporation of the solvent was done under a vacuum set at 140 mbar and temperature set at 45 °C until dry. Amber glass bottles were used for extract collection and placed in refrigerated conditions at 4–6 °C.

2.2. Ultrasound-Assisted Extraction (UAE)

The sonication technique extracted polyphenols from banana peel powders following Bimakr et al. (2013), with few amendments [26]. Preliminary studies were conducted to determine the optimum solvent concentration (25%, 50%, 75% and 100%), sample to solvent ratio (1:20, 1:15 and 1:10), and temperature (35 °C, 45 °C and 55 °C). After preliminary studies, three different solvents were used: acetone, methanol and ethanol at two different concentrations: 50% and 75%. Three different solvents, acetone, methanol, and ethanol, were used at two different concentrations: 50% and 75%. The sample to solvent ratio was set to 1:20, and the temperature for the sonication technique was set to 45 °C. About 3 g of peel powder were placed in 125 mL reagent bottles separately in triplicate, and 60 mL of solvent was added at 50% and 75% concentrations. A ‘Sonicator’ (Bandelin RK 510 H Sonorex, Heinrichstrabe, Berlin, Germany) was used in this procedure. The reagent bottles were placed in the sonicator bath for 1 h. Filtration of the samples was done with the assistance of Whatman filter paper 41, and centrifugation was performed for about 10–12 min at 5000 rpm (Beckman J2–21, Brea, CA, USA). Then the solvent was evaporated with a ‘Rotary Evaporator’ (Buchi Labortechnik, Rotavapor R-300, Meierseggsstrasse, Flawil, Switzerland). The solvent was evaporated under a vacuum set at 140 mbar and temperature set at 45 °C until dry. The extracts were collected in amber glass bottles and placed in refrigerated conditions at 4–6 °C.

2.3. Determination of % Yield

The following formula determined the % yield of the banana peel extracts:

$$\text{Yield (\%)} = \frac{\text{Weight of the Extract}}{\text{Sample Weight}} \times 100$$

2.4. Determination of Total Polyphenols (TPC)

The TPC of banana peel extracts was assessed using the Folin–Ciocalteu method described by Singleton et al., (1999) [27]. Ethanol, methanol and acetone solution sample extracts of 10 mg/mL were prepared. At 50% and 75% concentrations of solvents (ethanol, methanol and acetone), 0.5 mL solvent extract solution was mixed with 2.5 mL of 10% Folin–Ciocalteu’s reagent dissolved in water and 2.5 mL of 7.5% sodium carbonate. Blank was concomitantly prepared. The samples were then incubated at 25 °C for 30 min for blue color development. The absorbance was determined at 765 nm with a UV-a visible spectrophotometer. Gallic acid standard solution and the calibration curve were prepared from various concentrations (12.5, 25, 50, 100, 200 and 400 µg/mL) of Gallic acid. TPC was represented as mg gallic acid equivalent per g of extract (mg GAE/g, GAE = Galic Acid Equivalent).

2.5. Determination of Total Flavonoid (TFC)

The TFC of banana peel was determined following Chang et al., (2002) with few amendments [28]. 0.5 g sodium nitrate was dissolved in 10 mL of distilled water in a 10 mL volumetric flask to prepare a 5% sodium nitrate solution. 1 g of aluminum chloride was mixed in up to 10 mL distilled water in a 10 mL volumetric flask to prepare a 10% aluminum chloride solution. 1 g of NaOH was dissolved in 25 mL distilled water in a 25 mL volumetric flask to prepare 1 M NaOH solution. After the preparation of 3 different solutions, samples were prepared by taking 0.01 g of extract (banana peel) which was then dissolved in 5 mL of the respective solvent (methanol, ethanol and acetone). After that, 1 mL of sample extract solution was placed in a 10 mL volumetric flask and 4 mL of distilled water was added. Then 0.3 mL of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added. After 6 min, 2 mL of 1 M NaOH was added. The total volume was made up to 10 mL with distilled water. After that, absorbance was checked at 415 nm on a UV-visible spectrophotometer. Results were expressed as mg quercetin equivalent per g of extract (mg QE/g of section, QE = Quercetin Equivalent).

2.6. Evaluation of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of banana peel extracts was determined by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay following Brand-William et al., (1995) with slight amendments [29]. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at 20 °C until needed. A spectrophotometer obtained the working solution using a DPPH solution with methanol to absorb about 0.980 at 517 nm. 3 mL of that solution was mixed with 100 µL of the samples at varying concentrations (12.5–400 µg/mL). The test tubes were then shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The scavenging activity was estimated based on the percentage of DPPH radical.

2.7. Anti-Microbial Activity

The antimicrobial activity of banana peel extracts was determined by employing the method used by Greenwood [30]. Different bacterial species, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*, were assessed to measure growth inhibition. The media was prepared in distilled water, sterilization was carried out in an autoclave at 121 °C for 15 min and the media was cooled at room temperature after sterilization. Extracts of all the solvents at different concentrations were applied to sterile discs. Bacterial cultures were injected into sterilized discs and were then set for incubation.

2.8. Statistical Analysis

Data were statistically analyzed applying Analysis of Variance (ANOVA), Post Hoc Multiple Comparison, Fisher Least Significant Difference Test (LSD) and descriptive statistics. Then the results were converted into charts and tables (as appropriate) for better interpretation. Minitab 18.1 was used for analysis.

3. Results

3.1. Weight of Peels

Figure 1 illustrates the weight of banana peel at different stages, from fresh to oven-dried and powder. A substantial reduction in the weight was observed during the drying and grinding of fresh peels to powder form. The peel's weight reduction could be because of moisture loss and loss of sample while grinding dried peels into powder form.

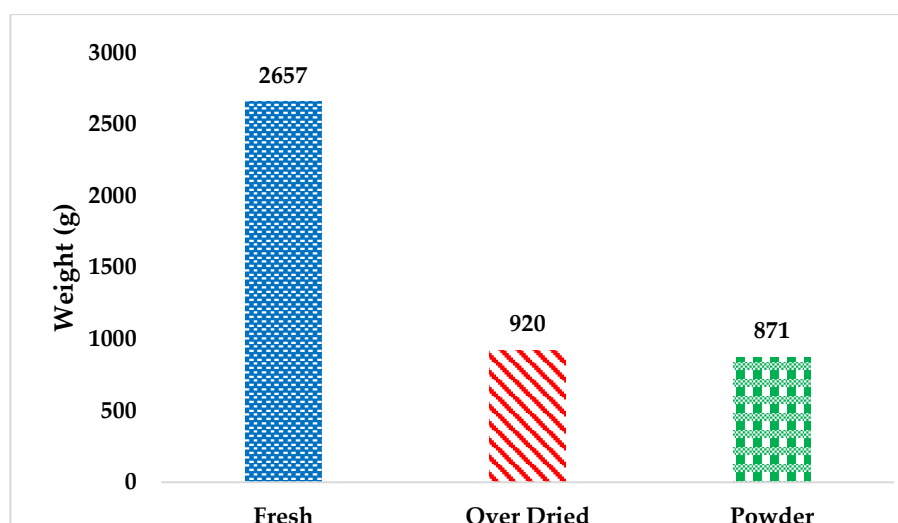


Figure 1. Weight of banana peels at different stages.

3.2. Proximate Analysis

Estimation of proximate composition is significant for assessing the quality of raw material [2,23]. Table 1 illustrates the proximate composition of banana peels. The variation in the proximate composition of banana peel powder in different studies could be because of different varieties, climatic conditions, topographic locations and agronomic practices.

Table 1. Proximate Composition (%) of Banana Peels.

Parameter	Moisture	Ash	Crude Fat	Crude Fiber	Crude Protein	NFE
Banana Peel	9.7 ± 0.06	6.67 ± 0.08	2.1 ± 0.03	17.51 ± 0.09	9.21 ± 0.05	54.8 ± 0.12

3.3. Yield (%)

Figure 2 illustrates the yield (%) of banana peel obtained using two different techniques (sonication and maceration) with three solvents (acetone, methanol and ethanol) at two different concentrations (50% and 75%). The variations in yield extractions using different solvents at different concentrations could be because of dissimilar polarities of the solvents used. Ethanol 50% was the best treatment for extraction as the yield was 13.48%. Similar results were obtained by Ranjha et al. [2] in apple and pomegranate peels and Safdar et al. [23] in the case of mango peel.

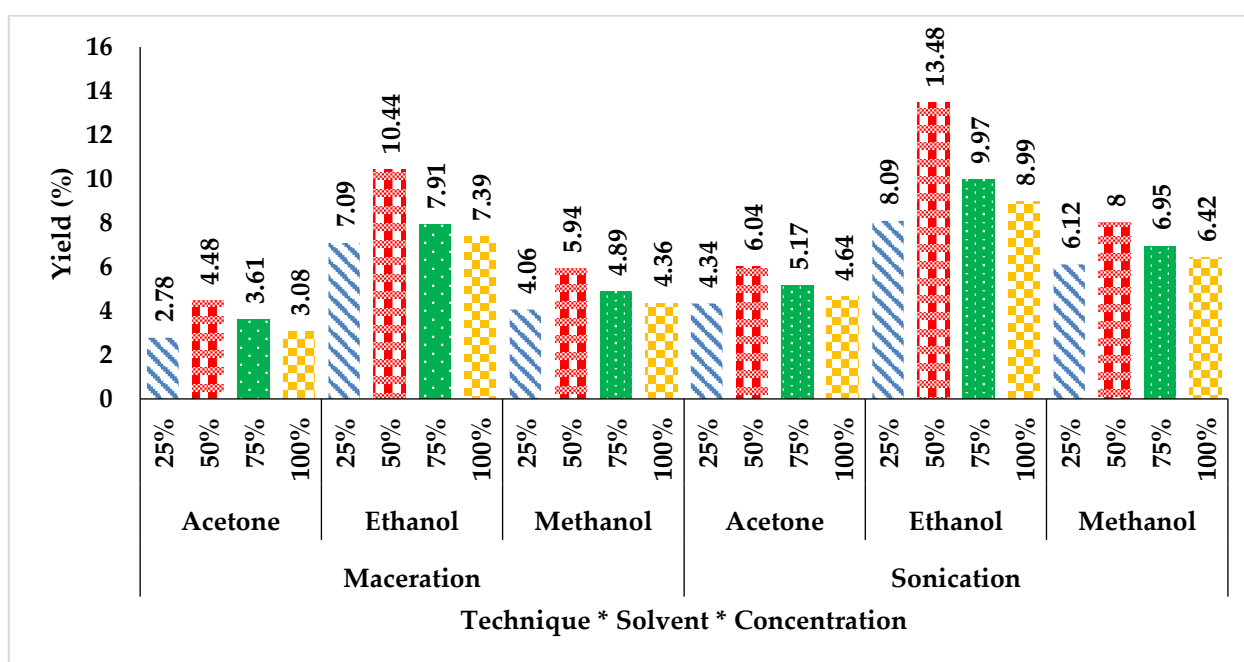


Figure 2. Yield (%) of banana peel extracts by sonication and maceration.

3.4. Total Polyphenolic Content (TPC)

Figure 3 illustrates total phenolic content as mg GAE/g. The highest phenolic concentration was found with ethanol 50% and was 31.46 mg GAE/g. It was also observed that phenolic content increased with an increase in ethanolic concentration up to ethanol 50% and decreased afterward. Very similar results were obtained in the case of acetone and methanol. It was also observed that sonication leads to better extraction than the maceration technique. Similar results were obtained by Ranjha et al. [2] in apple and pomegranate peels and Safdar et al. [23] in the case of mango peel.

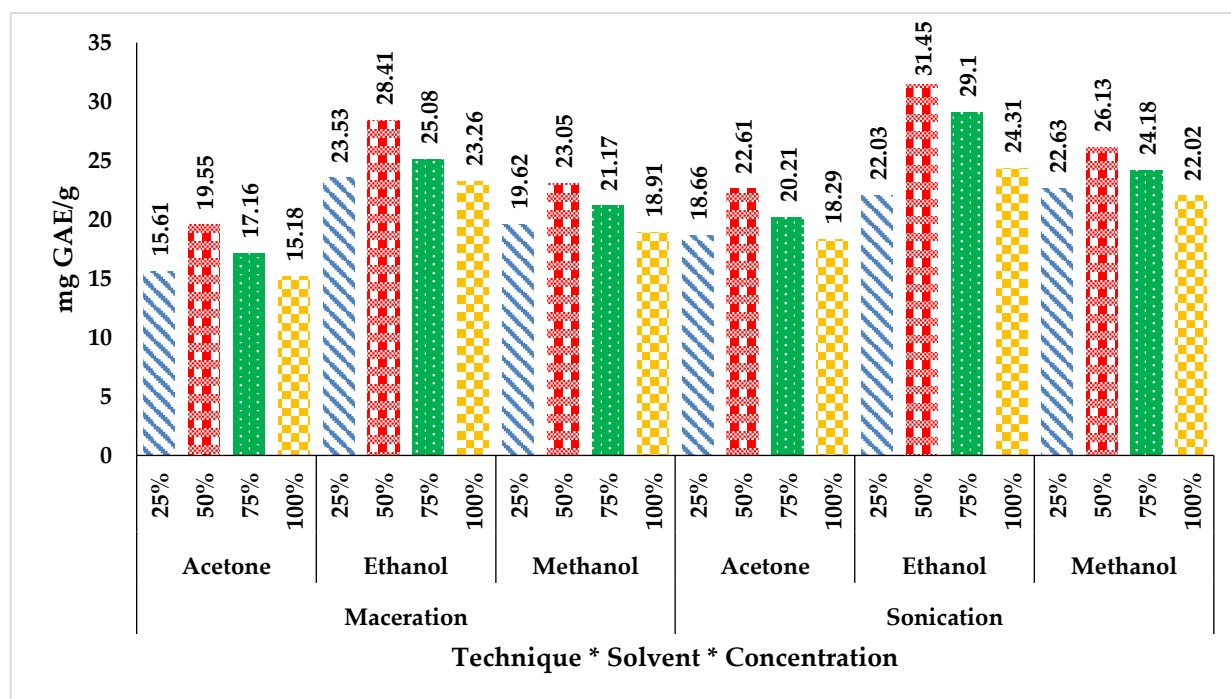


Figure 3. TPC of banana peel extracts.

3.5. Total Flavonoid Content (TFC)

Figure 4 illustrates total flavonoid content as mg QE/g. The highest phenolic concentration was found at ethanol 50% and was 22.11 mg QE/g. It was also observed that flavonoid content increased with increased ethanolic concentration up to ethanol 50% and decreased afterward. Very similar results were obtained in the case of acetone and methanol. It was also observed that sonication leads to better extraction than the maceration technique. Similar results were obtained by Ranjha et al. [2] in apple and pomegranate peels and Safdar et al. [23] in the case of mango peel.

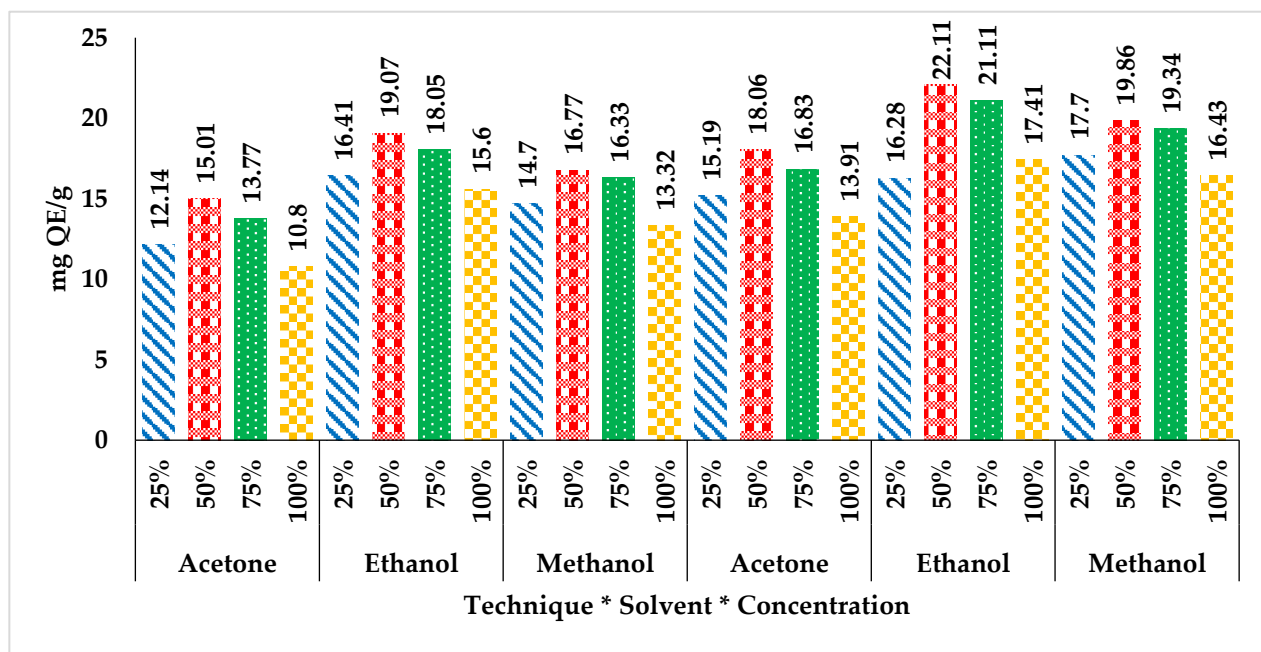


Figure 4. TFC of banana peel extracts.

3.6. Total Flavonoid Content (TFC)

All the banana peel extracts obtained were evaluated for their antioxidant potential. The results of the analysis are given in Table 2. It can be seen from the table that the highest antioxidant potential was observed for ethanol 75%, both in sonication and maceration. However, a more significant antioxidant potential was observed for extracts obtained employing sonication.

Table 2. Antioxidant Potential of Banana Peel Extracts.

Technique	Solvent		DPPH	FRAP
Sonication	Acetone	25%	67.69	23.26
		50%	76.8	26.83
		75%	73.2	26.03
		100%	69.68	24.91
	Ethanol	25%	75.4	26.11
		50%	82.5	28.5
		75%	80.1	27.56
		100%	77.17	26.95
	Methanol	25%	66.99	23.8
		50%	72.6	27.36
		75%	71.88	24.91
		100%	71.33	25.21
Maceration	Acetone	25%	63.26	19.59
		50%	73.82	22.96
		75%	70.75	22.6
		100%	65.01	22.81
	Ethanol	25%	72.11	24.1
		50%	78.91	26.31
		75%	77.78	25.3
		100%	74.92	24.58
	Methanol	25%	64.96	19.38
		50%	68.48	24.17
		75%	68.37	20.57
		100%	68.79	19.44

Similar results were obtained by Ranjha et al. [2] in apple and pomegranate peels and by Safdar et al. [23] in the case of mango peel.

3.7. Antimicrobial Activity of Banana Peel Extracts

The antimicrobial activity of banana peel powder extracts by both techniques was evaluated by well diffusion assay against different microbial isolates, including *S. aureus*, *P. aeruginosa*, *E. coli* and *S. cerevisiae*. The preliminary studies extract of 50% concentration was selected for all the microbial analyses. The obtained results are presented in Table 3 and show that Ethanol 50% extracts by sonication performed the most inhibition of bacterial species at 600 ppm against gram-positive bacteria such as *S. aureus* (11.31 mm), *P. aeruginosa* (12.11 mm), *E. coli* (15.43 mm) and *S. cerevisiae* (13.21 mm). Furthermore, it was noted that sonication resulted in better inhibition than the maceration technique, and overall, ethanolic extracts presented the best results of all the solvents.

Table 3. Antimicrobial Activity of Banana Peel Extracts.

Technique	Extract	Conc. µg/mL	Inhibition Zone (mm)			
			<i>St. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. cerevisiae</i>
Sonication	Acetone (50%)	200	0	0	0	0
		400	7.84	8.95	9.69	8.49
		600	10.47	11.02	14.42	12.51
	Ethanol (50%)	200	0	0	0	0
		400	8.45	9.77	10.22	9.56
		600	11.31	12.11	15.43	13.21
	Methanol (50%)	200	0	0	0	0
		400	6.87	7.9	8.77	7.46
		600	9.87	10.32	13.36	11.92
Maceration	Acetone (50%)	200	0	0	0	0
		400	5.78	6.45	6.19	2.99
		600	7.62	5.72	9.42	9.31
	Ethanol (50%)	200	0	0	0	0
		400	4.7	6.77	7.32	5.76
		600	8.07	9.21	11.23	9.71
	Methanol (50%)	200	0	0	0	0
		400	3.42	2	4.67	3.76
		600	6.9	7.62	8.46	6.024

The better results by sonication and ethanolic extracts may be due to better extraction of bioactive compounds from banana peel powder compared to other techniques and solvents. Similar results were obtained by Aboul-Enein et al. [31] in banana peel and Safdar et al. [32] in kinnow peel powder.

4. Conclusions

Banana peels are a rich source of phenolic compounds with significant antioxidant and antimicrobial activity. The current study concludes that better extraction could be done by employing ultrasound/sonication techniques in a very short time compared to the conventional maceration technique. Sonication results in better yield in a very short time economically. Moreover, the current study also concludes that the use of absolute solvent could not ensure fair extraction and it was observed that solvents at lower concentrations perform better extraction activity. Ethanol at 50% concentration results in a better product than other concentrations, and ethanol leads to better extraction than other solvents, i.e., acetone and methanol. The authors recommend that future studies should be planned to assess the influence of other innovative techniques on the extraction of bioactive compounds from banana peel powder.

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